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APPLICATION NUMBER: ***60/104,118***
FILING DATE: ***October 13, 1998***

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Docket Number: 1772/44761PV

Type a plus sign (+) inside this box -	+

INVENTOR(S)/APPLICANT(S)

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TITLE OF THE INVENTION (280 characters max)

Hydrocolloid Coating of Embryos

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ SPECIFICATION	NUMBER OF PAGES 4	☐ SMALL ENTITY STATEMENT
☒ DRAWINGS	NUMBER OF SHEETS 2	☐ OTHER (specify)

METHOD OF PAYMENT *(check one)*

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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Respectfully submitted,

SIGNATURE _____ Date October 13, 1998

TYPED or PRINTED NAME Richard R. Diefendorf REGISTRATION NO. 32,390 *(if appropriate)*

☐ Additional inventors are being named on separately numbered sheets attached hereto

PROVISIONAL APPLICATION FILING ONLY

Hydroc llold coating of embry s

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Faculty of Agricultural, Food and Environmental Quality Sciences

The Hebrew University of Jerusalem

Idea

Coating embryos by hydrocolloidal thin films to achieve:

a) Postpone hatching and extended survival rates.

b) Protection from microbial contamination

c) Protection from hazardous materials produced or introduced into the media.

d) As an inhibitor against damages occurred during freezing and thawing.

Example 1

X. laevis eggs were coated immediately after squeezing and fertilization by a thin layer (~50 μ) of film based on three different types of alginates varying in their mannuronic/guluronic ratios (Fig. 1a and b). The alginate was cross-linked either by Ca or Ba ions at three different concentrations. The development, survival and hatching of these embryos and the swelling of their natural jelly coats or hydrcolloid coating were studied during 7 days, while embryos were maintained either in flowing aerated water at a ratio of 85 ml per embryo or at a very diminished ratio of 0.6 ml sterile or non-sterile modified Marc Ringer's solution per embryo. All experiments were conducted in triplicates at 20±1°C. Oxygen was monitored continuously.

The coatings succeeded in postponing hatching in ca. 60 h in flowing aerated water at a ratio of 65 ml per embryo. However, the survival prospects diminished. Calcium as cross-linking agent was found to a better contribution.

However, major advantages of the coating were observed when the ratio between the embryos and the surrounding medium was maintained at its minimal value in non-sterile conditions, perhaps due to film resistance to diffusion. Here 0.25% barium and 0.25%calcium as cross-linking agents of the alginates gave the best results. In the studied systems, the coating seemed to postpone the hatching of the embryos. The difference in hatching time between the blank and the coated embryos was 30 to 60 h. In addition, the coating served as a barrier to microbial contamination and thus improved survival prospects. The number of microorganisms, counted directly at the medium after 5 days was 10^3 to 10^6 CPU, depends on conditions, medium temperatures and ratio between volume of medium and embryos number.

Example 2

Three different kinds of alginate with different gluronic (G) to mannuronic (M) acid ratios have been tried in coating the embryos. It is observed that the lower M to G ratio achieved better results with % of hatching. When this ratio was higher less penetration of high molecular weight compounds occur. Thus choosing the appropriate combination will determine the success of the coating.

Example 3

X. laevis eggs were coated immediately after squeezing and fertilization by a thin layer of films based on LMP (Low Methoxy Pectin), λ-carrageenan and

κ-carrageenan. The LMP was cross-linked either by Ca or Ba ions at different concentrations (other cross-linking ions are also possible). The λ-carrageenan and κ-carrageenan were cross-linked by Ca and potassium ions respectively at different concentrations. The development, survival and hatching of these embryos were studied during 7 days, while embryos were maintained at a ratio of 0.6 ml non-sterile modified Marc Ringer's solution per embryo. All experiments were conducted in triplicates at 20±1°C. For the λ-carrageenan and κ-carrageenan coated embryos, a higher survival rate than the non-coated embryos (calculated as a percent of the total hatching embryos) was observed. 1% LMP and 1% alginate were less effective. In fact, all of these coatings improved the survival rates under experiment conditions. The major advantages of the coating were observed when the ratio between the embryos and the surrounding medium was maintained at its minimal value in non-sterile conditions, perhaps due to film resistance to diffusion. In these systems, the coating seemed to postpone the hatching of the embryos. In addition, the coating served as a barrier to microbial contamination and thus improved survival prospects.

Example 4

Same as proposed for examples 1 and 3. The coated embryos were frozen by Planner cryo 10 at a rate <1°C/min up to a temp of-(-7°C) and then at a freezing rate >10°C/min to -50°C . The coated frozen embryos were transeferred to liquid nitrogen. The embryos were kept at each step for a few minutes for temperature stabilization, completing of crystallization and to permit majority of water to leave the cell. The percentages of embryo survival after one cycle of freezing and thawing were higher (in ~5 to 30%) than what that observed for the non-coated embryos. It is proposed that this result is the outcome of two

mechanisms. In the first, the coating served as a mechanical membrane and eliminates in part the penetration of the embryo by medium icicles. The second mechanism is probably the outcome of disturbance to ice to crystalize during the freezing.

Figs. Legends

Fig. 1a and b: embryo after fertilization and in advanced stage coated by alginate film.

Fig. 2a and b: emergence of embryos from the hydrocolloid coating

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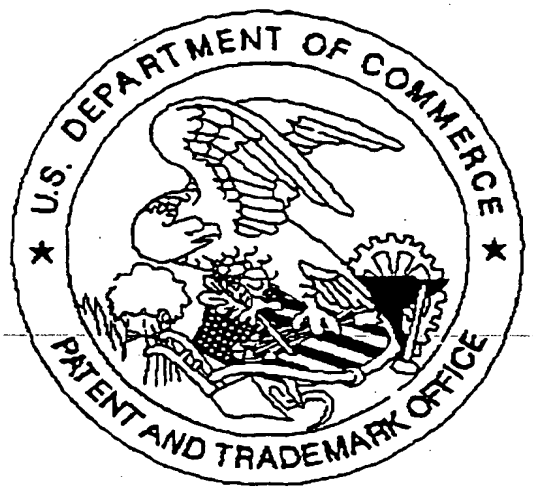

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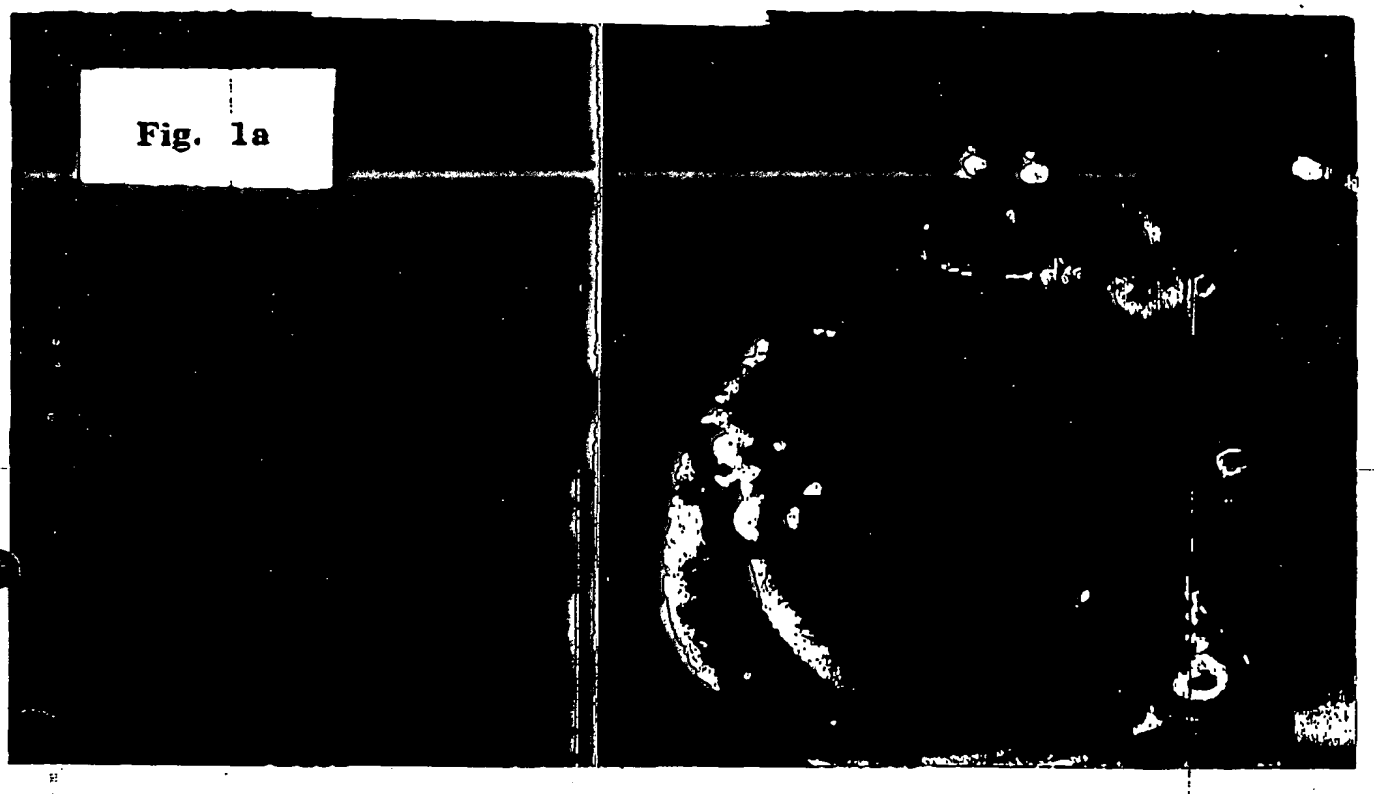

Fig. 1a

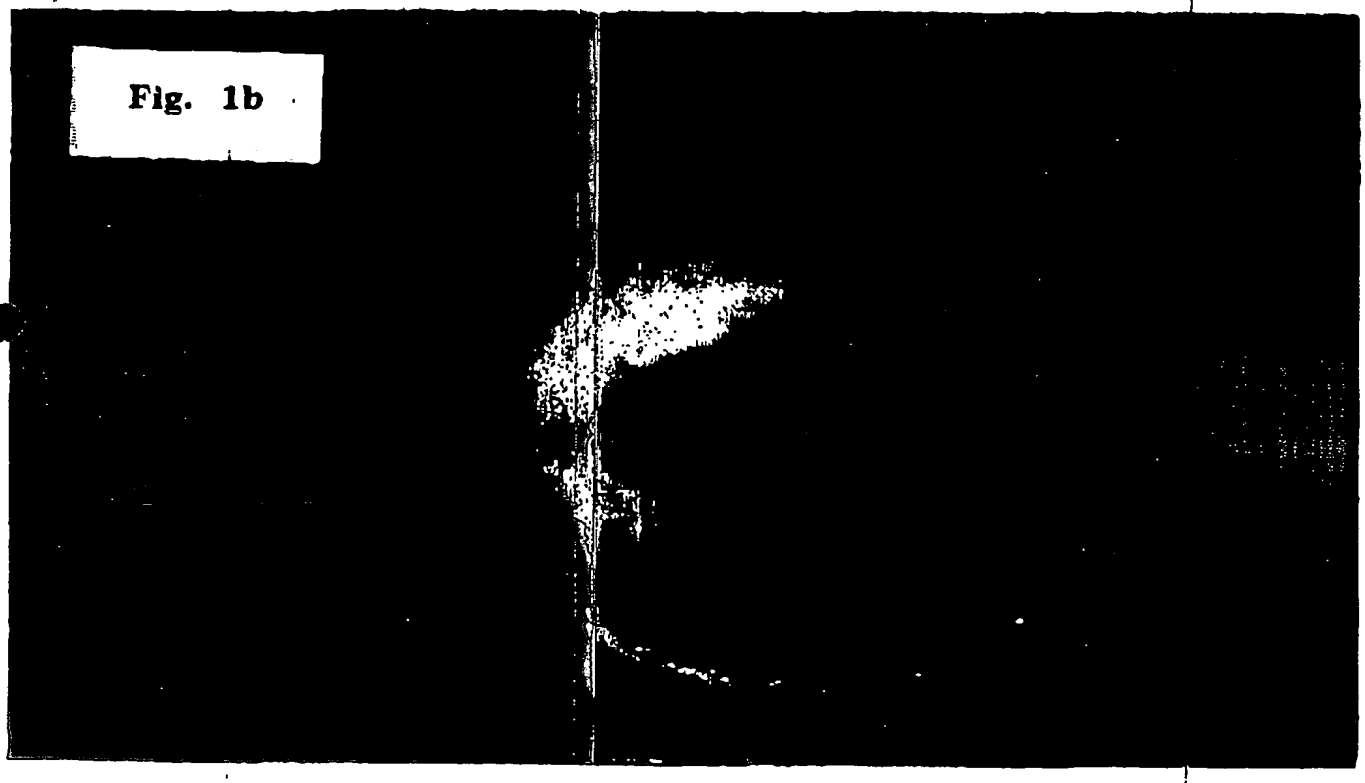

Fig. 1b

Fig. 2a

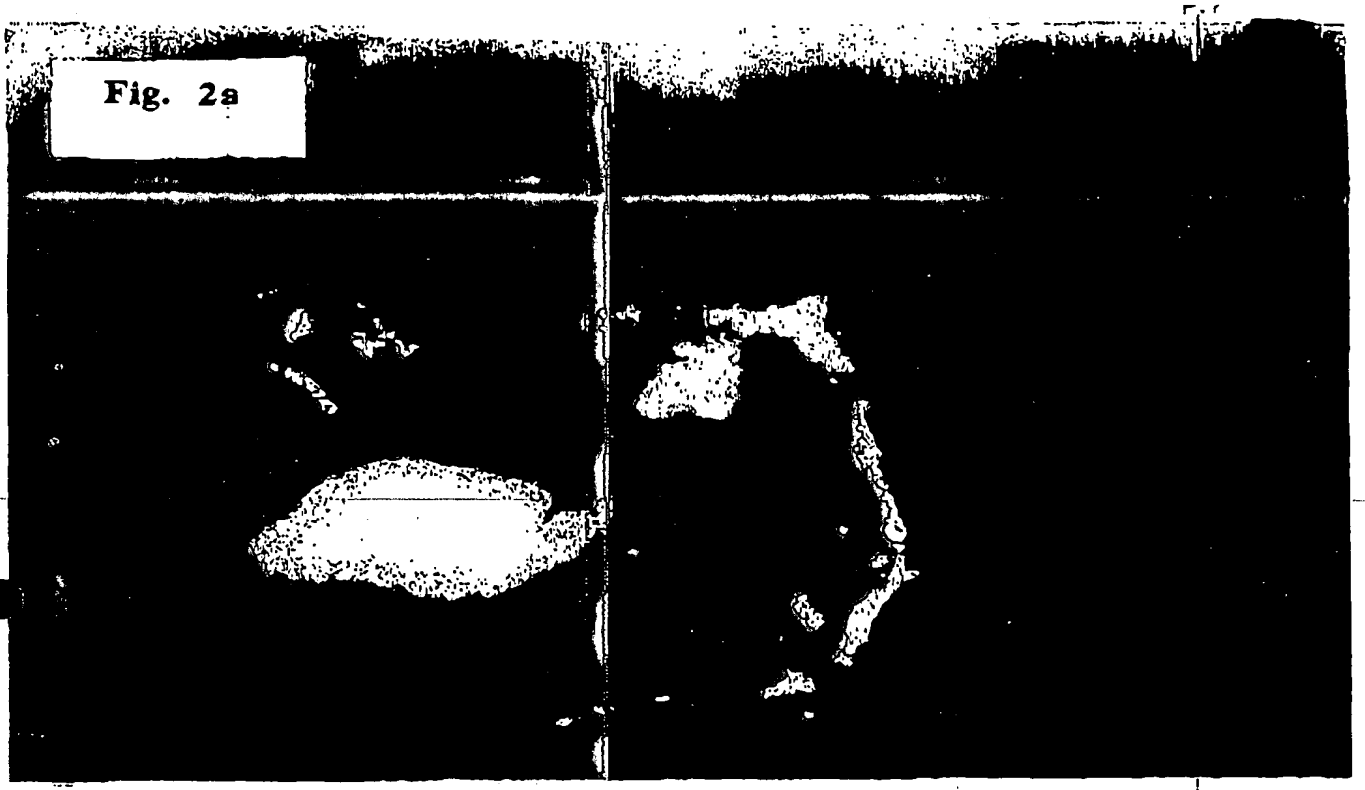

Fig. 2b